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Soy Isoflavone bioavailability - effects of probiotic and prebiotic consumption and oil supplementation

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CERTIFICATION

I, Theresa Anne Larkin, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Biomedical Science, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Theresa Anne Larkin

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DEDICATION

This thesis is dedicated to my admirable grandparents. The unconditional love that Nan, Pa, Pop and Nana have shown to so many and their unique and amazing characters are inspirational and beautifully motivating.

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ANOVA……analysis of variance
ANOVA/RM..……ANOVA with repeated measures
AUC……area under the curve
BMI……body mass index
CFU……colony forming units
C-max……maximum concentration
CVD……cardiovascular disease
D/G……daidzein: genistein ratio
DAID……. daidzein
DHA……docosahexaenoic acid
ECD……electrochemical detector
EPA……eicosapentaenoic acid
EQL…….....equol
GEN……..... genistein
HPLC……..high performance liquid chromatography
HRT……hormone replacement therapy
n-3……...omega-3
ODMA……o-desmethylangolensin
OO……..olive oil
PEP……p-ethyl phenol
PUFA……..polyunsaturated fatty acids
SEM……standard error of the mean
RS……...resistant starch
YC……... control yoghurt
YP……...probiotic yoghurt

ABSTRACT

Epidemiological evidence suggests a beneficial effect of soy consumption in relation to cardiovascular disease and in 1999, the U.S. Food and Drug Administration approved a health claim for the cholesterol-lowering effects of soy protein. However, this effect has not always been reproduced in subsequent soy dietary interventions, the results of which vary greatly. Soy beans are the richest dietary source of the isoflavones daidzein, genistein and glycitein, which have also been implicated with a role in lipid-lowering due to their antioxidant and estrogen receptor activities. After soy intake, there is large variability between individuals in isoflavone bioavailability. The isoflavones are present in soy as glycoside conjugates and after endogenous hydrolysis, the aglycones are absorbed, metabolised by liver and intestinal enzymes, distributed to tissues and excreted in urine. Endogenous isoflavone metabolites have been identified; of particular interest is the metabolism of daidzein to equol, as this has greater antioxidant and estrogen receptor activities than daidzein. Gut microflora is essential for isoflavone bioavailability and metabolism and can be affected by dietary modification. Differences in gut microflora composition may contribute to the large inter-individual variability in these processes, which in turn may contribute to variation in the lipid effects of soy consumption. Recently there has been much interest in the identification of dietary components that may enhance soy isoflavone bioavailability and it was the aim of this thesis to examine the effects of soy foods and other dietary components on soy isoflavone bioavailability and lipids. Four human studies were conducted and isoflavone bioavailability was determined based on plasma and urinary isoflavone levels. These samples were extracted with *Helix Pomatia* juice containing β-glucuronidases and sulphatases and the isoflavone aglycones were quantified by HPLC with electrochemical detection.

Resistant starch is a prebiotic and therefore has specific effects on gut microflora activity in the gastrointestinal tract and it was hypothesised that resistant starch intake may also affect isoflavone bioavailability. A pilot study with nine females (7 Australian and 2 Kenyan) was conducted to determine the acute and chronic effects of resistant starch

intake on soy isoflavone bioavailability. When resistant starch was consumed in the same meal as soy, plasma levels of daidzein and genistein were significantly reduced. However, daily resistant starch intake for 2 and 4 weeks prior to a soy meal resulted in a trend of increased mean daidzein excretion and of increased equol production in the two Kenyan subjects. It was concluded that resistant starch may enhance equol production, possibly dependent on gut microflora, genetics or habitual diet.

To determine whether there was an association between isoflavone bioavailability and lipid changes after soy intake, a soy dietary intervention was conducted in 23 hyperlipidemic men and postmenopausal women. Plasma and urinary daidzein and genistein levels were increased significantly after 6 weeks of soy milk and yoghurt intake. This treatment did not significantly affect lipids and there were no correlations between plasma or urinary isoflavone levels and lipid changes. However, in 8 subjects who produced equol in their plasma or urine, soy intake resulted in significant reductions in total and LDL cholesterol. This suggests that metabolism of daidzein to equol may be a determinant of the lipid-lowering effects of soy, contributing to this variation.

Based on the findings of the first two studies, a dietary combination of soy (cereal and milk) with either a probiotic or a prebiotic was proposed for further examination of the effects of resistant starch and equol production on isoflavone bioavailability and lipid levels. In a study of crossover design with 5-week dietary periods, soy consumption was compared with intake of soy plus either probiotic yoghurt or resistant starch-enriched bread for the effects on plasma and urinary isoflavone levels after a test soy meal in 31 hyperlipidemic men and postmenopausal women. Soy intake significantly increased circulating plasma daidzein and genistein levels, but did not affect plasma or urinary isoflavones after the test soy meal. There were no additional significant effects of either probiotic or prebiotic treatments; however, there was a trend for increased circulating plasma daidzein and genistein with probiotic treatment and for increased plasma daidzein and genistein 24 hours after the test soy meal with prebiotic treatment. Probiotic or prebiotic treatment did not induce or increase equol production, though there was a trend for increased plasma equol in “equol-positive” subjects (n = 12) after probiotic treatment.

The lack of any overall significant effects on isoflavone bioavailability with either probiotic or prebiotic treatment suggests that even if gut microflora was modified, this was not favourable for isoflavone bioavailability or equol production and thus it appears that other inherent features may determine these processes. Total cholesterol was significantly decreased with soy plus probiotic or prebiotic intake (-4.7 ± 2.0% and -5.5 ± 1.6% respectively) and LDL cholesterol was significantly decreased with soy intake and with prebiotic treatment (-4.1 ± 2.1% and -7.3 ± 2.2% respectively). This suggests that even in the absence of effects on isoflavone bioavailability, there was synergistic action between soy and probiotic or prebiotic intake for lipid-lowering effects and thus combination of these dietary components may be useful in lipid management.

For further examination of potential lipid-lowering effects of soy in synergy with other dietary components, it was hypothesised that a novel combination of soy with DHA-rich oil may also affect isoflavone bioavailability and result in a more positive lipid profile than supplementation with either component alone. DHA supplementation has strong triglyceride lowering effects, but it often also results in elevated LDL cholesterol, whereas conversely, a reduction in LDL is the most commonly reported lipid effect of dietary soy intake. In a crossover study with 35 hyperlipidemic men and postmenopausal women, plasma and urinary isoflavones were significantly increased after 6 weeks of soy (cereal) intake, but there were no significant effects of DHA-rich oil supplementation. Soy intake did not result in any significant lipid effects; however DHA supplementation resulted in a significant increase in HDL and decrease in triglycerides, independent of concurrent soy intake. In addition, there was an influence of the combination of DHA and soy compared with DHA alone for total and LDL cholesterol. While total and LDL cholesterol were increased with DHA supplementation alone, significantly for LDL, these increases were somewhat attenuated with concurrent soy intake. This suggests the potential for a combination of soy and n-3 fatty acids in producing lipid effects protective in relation to cardiovascular disease.

When the latter three studies were compared, a relation between the daidzein and genistein levels of food with the ratios of these isoflavones in plasma and urine was

evident, however some effects of soy food matrix were observed. Soy milk intake resulted in greater genistein bioavailability than daidzein compared to their relative amounts in the soy milk, while resistant starch intake appeared to increase daidzein excretion more than genistein. Furthermore, there was a particular finding of high occurrence of equol in plasma in the third study examining the combination of soy with oil; this was suggested to be due to the higher proportions of daidzein and glycitein than genistein in the soy germ product ingredient of the cereal.

Overall, there appears to be the potential for probiotic and prebiotic foods and the soy matrix isoflavone composition to influence soy isoflavone bioavailability. These findings are important in relation to physiological activities of soy foods as the isoflavones differ in their bioactivity and require further investigation. Further, in relation to lipid effects of soy consumption, baseline levels of total and LDL cholesterol were both significantly inversely correlated with subsequent lipid changes with soy intake. In addition, there were beneficial additive hypocholesterolemic effects of soy with probiotic and prebiotic foods and with DHA-rich oil supplementation. In conclusion, the bioavailability of isoflavones from soy is affected by other dietary components and the soy matrix in which they are contained. This did not appear to influence lipid effects which were modest and only significant when soy was consumed concurrently with other dietary components also known to produce beneficial effects. The latter finding has application in the development of functional foods for those with elevated lipids.

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